

prevented Aquaporin1 mal-folding. Bioimaging of live yeast cells revealed that recombinant Aquaporin-1 accumulated in the yeast plasma membrane. A detergent screen for solubilization revealed that CYMAL-5 was superior in solubilizing recombinant Aquaporin-1 and generated a monodisperse protein preparation. A single Ni-affinity chromatography step was used to obtain almost pure Aquaporin-1.

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Aquaporin Trafficking as a Specific Regulatory Mechanism to Adjust Membrane Water Permeability

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IBBEA DBBE, Universidad de Buenos Aires, Buenos Aires, Argentina. Plant aquaporins regulate water fluxes across membranes by enhancing membrane water permeability (P_f). In particular, the plant plasma membrane holds PIPs, one of the largest groups of aquaporins. PIPs are divided in two clusters (PIP1 and PIP2) that disclose intriguing aspects: i) the potential of modulating P_f by PIP1-PIP2 co-expression, distinguished for each PIP showing differential capacity to reach the PM and ii), the faculty to reduce water permeation through the pore after cytosolic acidification, as a consequence of a gating process. Our working hypothesis is that cytosolic pH (gating) and PIP co-expression (trafficking) enhance plasticity to the membrane water transport capacity as a consequence of a PIP1-PIP2 cooperative interaction. Thus, PIP1 cellular trafficking and its effect in water permeability emerge as playing a key role as a regulatory mechanism. To analyze this interaction we used PIP1-PIP2 pairs from different species (*Fragaria ananassa* and *Beta vulgaris*). Our experimental approach included i) designing mutants to alter the PIP-PIP2 interaction by means of site directed mutagenesis; ii) tracking aquaporin localization -at internal structures or expressed at the level of the PM-; and iii) analyzing water transport capacity in control and inhibited (medium acidification) conditions by means of measuring P_f in *Xenopus* oocytes. Our finding support evidences in agreement with the concept that PIP2 and PIP1 interact to form functional heterooligomeric assemblies, and thus the composition of these PIP assemblies determines their functional properties. As PIP1 alone is not able to reach the plasma membrane its contribution to enhance water permeability is associated to its translocation and interaction with a PIP2. This regulatory mechanism seems to be present in different vascular plants. This information is integrated in a proposal for water transport pathways including the organs where this PIPs are present.

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Selectivity Filter Scanning of the Human Voltage Gated Proton Channel HhV1

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¹Molecular Biophysics & Physiology, Rush University, Chicago, IL, USA, ²ICS-4 Zelluläre Biophysik, Institute of Complex Systems, Jülich, Germany, ³Department of Biology and Physics, Kennesaw State University, Kennesaw, GA, USA, ⁴Molecular Structure and Function, Hospital for Sick Children, Toronto, ON, Canada, ⁵Department of Biochemistry, University of Toronto, Toronto, ON, Canada, ⁶Medicine, University of Chicago, Chicago, IL, USA. Extraordinary selectivity is crucial to all proton conducting molecules, including the human voltage gated proton channel, hHv1, because $[H^+]$ is minuscule. Here we use "selectivity filter scanning" to elucidate the molecular requirements for proton specific conduction in hHv1. Asp¹¹², in the middle of the S1 transmembrane helix, is essential to the WT channel selectivity [Musset et al., 2011. *Nature* 480:273-277]. We neutralized Asp¹¹² by mutating it to Ala (D112A), then introduced Asp at each position along S1 from 108 to 118, searching for "second site suppressor" activity. All mutants except for D112A/V109D lacked even the anion conduction exhibited by D112A. Proton specific conduction was restored with Asp or Glu at position 116. The D112V/V116D channel resembled WT in selectivity, kinetics, and ΔpH dependent gating. Both R211H and R211H/D112V/V116D were inhibited by internally applied Zn^{2+} when the channel was open, indicating similar S4 accessibility. At position 109 Asp allowed anion permeation in combination with D112A, but did not rescue function in the nonconducting D112V mutant, indicating that selectivity is established external to the constriction at Phe¹⁵⁰. The three positions (109, 112, 116) that permit conduction all line the pore in our homology model, delineating the conduction pathway. Evidently, proton selective conduction requires a carboxyl group to face the pore at a constriction in the external vestibule. Molecular dynamics studies indicate reorganization of ionic networks in response to mutations and suggest that the distribution of charged

groups in the external vestibule modulates charge selectivity. That the selectivity filter functions in a new location helps define local environmental features that produce proton selective conduction.

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The Permeation Pathway Mechanism in Ciona Intestinalis Hv Channel

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Voltage-gated proton (Hv) channels are expressed by different cells type including immune cells, microglia, among others. In other voltage-gated cation channels, the pore-forming domains are S5, S6, and the P loop connecting S5 and S6. Since Hv channels do not contain a S5-S6 region, other parts of the channel must form the pore domain. Interestingly, Hv channel present three gating charges in the S4 followed by an asparagine, N264, highly conserved among all Hv channel. Using non-stationary fluctuation analysis we establish the conductance for the dimer and the monomer in 200 and 100 fS, respectively. Mutations at position S191 (S2) and N264 (S4) modified the unitary conductance of Hv channel. Furthermore, mutants S191E/D and N264R removed the H^+ current remaining the S4 functioning unaltered, according to voltage-clamp fluorometry experiments. The introduction of an arginine or lysine at position 264 or negative residue at position S191, drastically reduced or abolished the proton currents. Interestingly, mutations of the analogue position S191 of Ciona VSOP, in the voltage sensor domain of a non-conductor Shaker K^+ channel produce fully functional voltage gated H^+ channels. In the same way, mutation resembling the position N264 of Ciona Hv in the VSD of the voltage-dependent phosphate Ci-VSP spontaneously entail the formation of a voltage-gated H^+ channel. Thus, S191 and N264 form the molecular determinants of permeation pathway in Hv channel. Supported by Fondecyt grants ACT 1104 and Fondecyt 1120802 to CG and 1110430 to R.L.

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Mechanism of Non-Selectivity in NAK Channel

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The amino acid sequence of the non-selectivity filter of the bacterial NaK channel (TVGDG) is similar to that of the K-selective KcsA channel (TVGYG), yet NaK conducts both Na^+ and K^+ equally well. To investigate the non-selectivity mechanism of NaK, we performed non-equilibrium molecular dynamics simulations using step-wise pulling protocols and Jarzynski's Equality. Two ions in the crystal structure of NaK were kept at site S3 ($z \approx 14.0$ Å) in the filter and in the small vestibule above S3, and one ion for pulling was placed at $z = 0$ Å below the filter entrance. Two sets of simulations with either NaCl or KCl were compared to quantify how the filter dehydrates and conducts the different ions along the z-axis toward the extracellular surface. The simulations show that the entry of Na^+ into the filter is favored by about 0.5-2 kcal/mol over K^+ due to easier dehydration of Na^+ . This difference is attributed to the collapse of S3 by 11% in volume on Na^+ , resulting in a higher negative charge density of carbonyl oxygen atoms around Na^+ than K^+ . When ions enter the filter and displace the ion at S3 (called Na3 or K3) into the small vestibule, the pulled K^+ in S4 (below S3) is more dehydrated than the pulled Na^+ . K3 and the other K^+ and water molecules in the filter become significantly more symmetric around the z-axis than Na^+ and water molecules. This symmetric distribution of K^+ ions and water favors the movement of K^+ above S3, offsetting the slight ion selectivity at the filter entrance. We hypothesize that the non-selectivity filter of the NaK channel favors Na^+ over K^+ below S3, but becomes more selective for K^+ than Na^+ above S3, thus resulting into the non-selectivity as experimentally observed.

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Statistics of Simulated Ion Channels

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The statistical analysis of ionic currents through biological ion channels is straightforward when single channels are recorded. However, analysis can be intractable when more than one channel is present due to signal overlap. We present a statistical analysis of simulated ion channel recordings when the number of channels is small. Through numerical analysis, we display relationships